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Running title: Adult effects of fetal IGF1 therapy for FGR

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Abstract

Fetal growth restriction (FGR) is associated with compromised growth and metabolic function throughout life. Intrauterine therapy of FGR with intra-amniotic insulin-like growth factor-1 (IGF1) enhances fetal growth and alters perinatal metabolism and growth in a sex-specific manner, but the adult effects are unknown. We investigated the effects of intra-amniotic IGF1 treatment of FGR on adult growth and body composition, adrenergic sensitivity, and glucose-insulin axis regulation. Placental embolisation-induced FGR was treated with 4 weekly doses of 360 µg intra-amniotic IGF1 (FGRI) or saline (FGRS). Offspring were raised to adulthood (18 months: FGRI, *n*=12 females, 12 males; FGRS, *n*=13 females, 10 males) alongside offspring from un-embolised and untreated sheep (CON; *n*=12 females, 21 males). FGRI females had increased relative lean mass compared with CON but not FGRS ($p<0.05$; 70.6±8.2% vs. 61.4±8.2% vs. 67.6±8.2%), decreased abdominal adipose compared with CON and FGRS ($p<0.05$; 43.7±1.2% vs. 49.3±0.9% vs. 48.5±1.0%), increased glucose utilisation compared with FGRS but not CON ($p<0.05$; 9.6±1.0 vs. 6.0±0.9 vs. 7.6±0.9 $mg \cdot kg^{-1} \cdot min^{-1}$), and increased β-hydroxybutyric acid:non-esterified fatty acid ratio in response to adrenaline compared with CON and FGRS ($p<0.05$; 3.9±1.4 vs. 1.1±1.4 vs. 1.8±1.4). FGRS males were smaller and lighter compared with CON but not FGRI ($p<0.05$; 86.8±6.3 vs. 93.5±6.1 vs. 90.7±6.3 kg), with increased peak glucose concentration (10%) in response to a glucose load, but few other differences. These effects of intra-amniotic IGF1 therapy on adult body composition, glucose-insulin axis function, and adrenergic sensitivity could indicate improved metabolic regulation during young adulthood in female FGR sheep.

Keywords: Fetal therapy, intrauterine intervention, fetal programming, insulin sensitivity, adrenergic sensitivity, metabolism

Introduction

Fetal deprivation results in altered *in utero* metabolic function that subsequently increases the risk of adult metabolic dysfunction (40). Fetal growth restriction (FGR), a failure to attain intrauterine growth potential (21), overlaps, but is not synonymous with, small-for-gestational age (SGA), a statistical definition of growth (usually birthweight ≤10th centile). The SGA neonate has increased peripheral insulin sensitivity at birth (15, 25) and glucose disposal at 48 hours of age (2). However, small size at birth and thinness often are followed by accelerated postnatal growth (3). This growth pattern, even over the first year after birth, is associated with decreased insulin sensitivity at one year (49) and increased visceral adiposity in later childhood (10, 42, 48), and early adulthood (30).

Metabolic dysfunction and rapidly increasing fat deposition increase the risk for non-communicable diseases (NCDs) in adulthood (20). Decreased insulin sensitivity in adults with low birth weight is reportedly due to decreased insulin secretory capacity (28), insulin-stimulated glucose uptake (23), and insulin-signaling in skeletal muscle (27, 45). Studies in sheep suggest that FGR also leads to pancreatic β-cell dysfunction (22).

FGR has been well studied in sheep, with substantial data indicating physiologic and molecular mechanisms of adaptation during fetal life (38). These studies suggest that the metabolic effects of FGR seen in postnatal life have their origins before birth, including decreased pancreatic β-cell mass and insulin secretion (32, 33). Thus, the optimal time for an intervention to prevent the long-term metabolic sequelae of FGR may be before birth. However, there are currently no effective clinical therapies for FGR. Therapies with potential from animal studies include maternal sildenafil citrate (16, 44) and intra-amniotic insulin-like growth factor (IGF)-1 (5, 6, 11, 17, 51, 53).

Weekly intra-amniotic IGF1 injection (360 μg·d^{-1}) from 110-124 days' gestation (term 145) increases fetal growth trajectory and mRNA expression of placental amino acid transporters in the FGR sheep without any adverse perinatal effects (51, 53). We previously have reported that growth-altering effects of this brief fetal intervention persist into the immediate postnatal period in a sex-specific manner (51). However, the long-term effects through to adulthood are unknown. Thus, the aim of this study was to investigate the effect of intra-amniotic IGF1 treatment of the growth-restricted ovine fetus on growth, body composition and metabolism from the early postnatal period through to early adulthood (18 months). We hypothesised that intra-amniotic IGF1 treatment would normalise postnatal growth, body composition and metabolism compared with saline-treated control fetuses and that effects would differ in a sex-specific manner.

Materials and Methods

All experimental procedures were approved by the University of Auckland Animal Ethics Committee (AEC/02/2008/R628 and AEC/03/2011/R874). Reporting of this experiment conforms with the ARRIVE Guidelines (29). The primary outcome for the experiment was glucose tolerance at 18 months of age. Based on previous studies in young adult sheep, we calculated that to detect a change in mean glucose area under the curve in a glucose tolerance test of 15% from 712 (SD 99) mmol·min^{-1}·L^{-1}, with 80% power and a 2-tailed significance level of 0.05, 12 successful studies were required per experimental group. Given that the outcomes being assessed (growth, body composition and glucose-insulin axis function) are known to differ between females and males, we determined *a priori* to analyse data separately by sex. We therefore required 12 successful studies per sex per experimental group.

Animals were produced as previously described (51). Briefly, FGR was induced in sheep fetuses from 103-107 dGA by bilateral maternal uterine artery embolisation, twice daily titrated to fetal blood gases. Randomly allocated FGR fetuses received either sterile saline (FGRS) or 360 µg (100 µg·mL^{-1}) recombinant human IGF1 (FGRI; Genentech, San Francisco, California) by weekly intra-amniotic injection 107-135 dGA. Numbers of pregnant ewes, fetal and perinatal losses resulting in the offspring numbers reported in this paper are detailed in the previous publication.

Animal husbandry. At two weeks of age, individually housed ewe-lamb pairs were transitioned into a mob. At three weeks of age a rubber ring was applied to dock the tail, and lambs were transferred with their dams onto pasture. Lambs were weaned and split into same-sex mobs at 3 months of age (males were not castrated) and maintained on pasture until 17-18 months of age (early adulthood, puberty is ~7-8 months). At 4, 12, and 17-18 months sheep were brought into the feedlot where they were housed individually but with direct contact with, and in the sight and sound of, other sheep.

Weight and linear measures. Weight, crown-rump length (CRL), abdominal circumference, and hock-to-toe length (HT), were measured at two and three weeks, and monthly thereafter. Growth velocity for weight and linear measures were calculated as described previously (51). Relative weight per unit body length was calculated as body mass index (BMI, kg·CRL^{-2}).

Blood sampling, hormone and metabolite analyses. Blood samples were collected via venipuncture at 2, 3 and 4 weeks, and at 2 and 3 months. Throughout the physiologic

testing procedures blood was collected via indwelling catheters (26). Whole blood glucose concentration was measured on a YSI 2300 (Yellow Springs Instruments, Queensland, AU). Blood samples were centrifuged at 3,220 *g*, 4°C for 10 minutes and plasma was stored at -80°C until biochemical analysis.

Plasma metabolite (glucose, non-esterified fatty acid (NEFA), urea and β-hydroxybutyric acid (βHBA)) concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High-Technologies Corporation, Tokyo, Japan) as described previously (51). Mean inter- and intra-assay coefficients of variation (CV), respectively, were 4.3% and 3.8% for urea; 3.4% and 2.4% for NEFA; 4.0% and 1.1% for βHBA, and the mean intra-assay CV for glucose was 1.6%.

Plasma insulin was measured by radioimmunoassay (RIA) with ovine insulin (Sigma-Aldrich, St. Louis, Missouri, USA) as the standard (41). The minimum level of detection was 0.03 $ng \cdot mL^{-1}$. Mean inter- and intra-assay CVs were 8.6% and 11.7%, respectively.

Dual X-ray absorptiometry (DXA). Animals were sedated for DXA scans (Norland, XR-800, Cooper Surgical Ltd, Fort Atkinson, WI, USA) at 4, 12 and 18 months of age. Sedation was induced with intravenous 5.0 $mg \cdot kg^{-1}$ ketamine and 0.25 $mg \cdot kg^{-1}$ diazepam (Parnell Co. Ltd. and Ceva Animal Health Ltd., respectively, Auckland, NZ) and maintained with 10.0 $mg \cdot kg^{-1}$ ketamine and 0.5 $mg \cdot kg^{-1}$ diazepam. Palpebral reflex and arousal state were monitored, and sedation titrated as needed.

At 4 months DXA scans were conducted as described previously (51). At 12 and 18 months, scans were undertaken at a spatial resolution of 6.0 x 6.0 mm. The area analysed was divided into three areas: chest; abdomen, and rump. The chest area was bounded by lines connecting the first cervical vertebra, the first lumbar vertebra,

the diaphragm, and the sternum and along the breast to include the heads of the radii and ulnae. The abdominal area was bounded by the chest area, the lumbosacral spine at the anterior line of the pelvis, along the anterior margin of the pelvic limb and around the ventral portion of the abdomen. The rump area was bounded by the abdominal area and included the base of the tail and the femorotibial joint. Fat and lean mass and bone mineral content were estimated using Norland software (Cooper Surgical Ltd, Fort Atkinson, WI, USA) and expressed in grams. Fat and lean mass of each area were calculated relative to both overall compartment mass and total bodyweight.

Physiologic testing. To ensure ewes were not in estrus at the time of study, estrus was synchronised with an intravaginal controlled internal drug release device (CIDR; Pfizer, Auckland, NZ) containing 0.03 g progesterone. Adult sheep were allocated to undergo adult physiologic testing using a random number generator. Sheep randomised not to receive tests were housed alongside those undergoing procedures throughout. Three days prior to testing, feed intake was titrated to allow 10% by weight feed refusal per day. Indwelling jugular venous catheters were inserted as described previously (26). and maintained with flushes of 10 $U \cdot mL^{-1}$ sodium heparinised 0.9% sterile saline (sodium heparin, Hospira, Victoria, AU). Sheep underwent an intravenous glucose tolerance test (IVGTT) and hyperglycemic clamp (HGC) following an overnight fast, and an epinephrine stimulation test (EPI) in the fed state. Animals were free-moving during the IVGTT and EPI, but were restrained within their pens using straps during the HGC, a necessary precaution due to the nature and duration of the test.

Intravenous glucose tolerance test. Following an overnight fast, 0.5 $g \cdot kg^{-1}$ 50% dextrose was infused in ≤60 seconds and followed by a 10 mL 0.9% saline flush. Blood samples were collected at baseline, and 2, 5, 10, 15, 20, 30, 40, 50, 60, 120 and 180 minutes post-bolus.

Hyperglycemic clamp. Two days after the GTT and following an overnight fast, blood samples were taken at -20, -15, -10 and 0 minutes to determine basal whole blood glucose concentration, followed by a 7.7 $mL \cdot minute^{-1} \cdot m^{-2}$ body surface area ($kg^{0.67}$ x 0.09 (36)) 25% dextrose bolus over 0-5 minutes to increase blood glucose concentration to 10 $mmol \cdot L^{-1}$. The rate of glucose infusion thereafter was adjusted according to a computer algorithm to maintain blood glucose concentration at a 10 $mmol \cdot L^{-1}$ steady state (SS). Blood samples were collected at 5-minute intervals through 135 minutes to determine whole blood glucose concentration; plasma was prepared from samples collected at 15-minute intervals for insulin assay. To maximize insulin secretion, a 100 $mg \cdot kg^{-1}$ L-Arginine bolus was infused immediately following the 135-minute blood sample (4) followed by blood sample collection at 5-minute intervals to determine whole blood glucose concentration and plasma prepared from samples collected at 5, 10, 20, and 30 minutes post-bolus for insulin assay. HGC data were analysed if the blood glucose concentration coefficient of variance was ≤10% during SS (75-135 minutes).

Analysis of glucose tolerance and insulin sensitivity. In the pre-weaning lambs from 2-12 weeks of age, insulin secretion and sensitivity were assessed with the homeostasis model assessment (iHOMA2) of β-cell function (HOMA-%β) and insulin resistance

(HOMA-%S) from plasma glucose and insulin concentrations (24), and absolute change from 2-12 weeks was calculated.

In adult sheep, glucose tolerance was assessed with IVGTT by calculating the areas under the curve (AUC) for glucose during the first (0-15 minutes) and second (15-180 minutes) phases after the glucose bolus (mmol·L^{-1}; IVGTT-AUC_{G1} and IVGTT-AUC_{G2}, respectively) (46), the first phase insulin response (AIR_{15}) (34, 43), change in insulin secretion from baseline in the first phase (ΔI_{S1}), the second phase AUC for insulin (IVGTT-AUC_{I2}) and insulin secretion relative to glucose during SS (I_S, ng·mL^{-1}·mmol·L^{-1}) (19).

Pancreatic β-cell function in the adult was assessed with HGC; insulin AUC (HGC-AUC_I) was calculated using the trapezoidal rule from baseline to the end of SS (HGC-AUC_{ISS}) and following the L-Arginine bolus to completion of the clamp (HGC-AUC_{IARG}). We calculated mean glucose infusion during SS (IG_{SS}: mmol·kg^{-1}·min^{-1}) and following the L-Arginine bolus (IG_{ARG}: mmol·kg^{-1}·min^{-1}), mean plasma insulin concentration during SS (I_{SS}: ng·mL^{-1}), peak plasma insulin concentration following the L-Arginine bolus (I_{ARG}: ng·mL^{-1}), the change in insulin secretion from SS in response to the L-Arginine bolus (ΔI_S ARG:SS), insulin sensitivity during SS (S_i SS= IG_{SS}/I_{SS}) and following the L-Arginine bolus (S_i ARG= IG_{ARG}/I_{ARG}) (37), the insulin-dependent component of whole-body glucose tolerance during SS, the glucose disposition index (DI_G = S_i SS x AIR_{15}) (34), and glucose-stimulated insulin secretion (Mean I_{SS}/basal plasma insulin) (7, 31).

Epinephrine stimulation test. After collecting a baseline blood sample, 1.0 µg·kg^{-1} epinephrine (Mayne Pharma, Salisbury South, South Australia, AU) was injected intravenously followed by a 10 mL 0.9% saline flush. Blood samples were collected at

2.5, 5, 7.5, 10, 15, 20, 30, 45 and 60 minutes. Adrenergic responsiveness was assessed by calculating the AUC for plasma glucose, βHBA, and NEFA in the first phase post-injection (first 7.5 minutes: EPI-$AUC_{G7.5}$, EPI-$AUC_{NEFA7.5}$, and EPI-$AUC_{\beta HBA7.5}$), peak response, and glucose and βHBA relative to NEFA response at 7.5 minutes.

Post-mortem. All post-mortems were conducted following an overnight fast. In sheep randomised to receive physiologic tests, post mortems were conducted 5-6 days after completion of testing to allow adequate time for recovery and drug elimination, and those of untested adult sheep were conducted in parallel. Live weight was collected, and sheep euthanised with a 100-120 $mg \cdot kg^{-1}$ sodium pentobarbitone injection. Total adrenal, brain, cerebellum, heart, total kidney, liver, total lung, pancreas, pancreatic lymph node, total perirenal fat, neck thymus, and thyroid weights were collected. Fat depth was measured at the 12^{th} rib on the left side of the carcass, (9), and empty carcass was weighed.

Statistical analysis. Data were analysed in JMP 12 (SAS Institute Inc., Cary, North Carolina, USA) and GraphPad Prism 7.0 (La Jolla, California, USA). Distribution was verified with the Shapiro-Wilk test. Non-normally-distributed data were log transformed to approximate a normal distribution where necessary. The effects of FGR and intra-amniotic treatment of FGR with IGF1 were analysed by factorial or repeated measures analysis of variance (ANOVA and RM ANOVA, respectively) in each sex with breeding year as a random effect. RM ANOVA was used to examine changes over time. Correlations were assessed by linear regression analysis separately for each sex.

Significance was set at $p<0.05$ unless otherwise stated. Tukey's *post hoc* testing was conducted where appropriate. Results are presented as mean ± SEM.

Results

Animal numbers and losses throughout the study. Ninety-eight sheep survived to two weeks of age and entered the study (Supplementary Figure 1) (51). Postnatal mortality was low and not different amongst groups (Supplementary Table 1). For perinatal outcomes, see Spiroski, 2017. Briefly, FGR lambs were ~20% lighter at birth, and IGF1 treatment partially reversed the reduced size at birth in females, and reduced FGR-mediated acceleration in growth velocity in both sexes.

Pre-weaning linear growth. In females, FGRI had reduced CRL compared with CON at weaning ($p<0.05$) and decreased pre-weaning GV of CRL (FGRI 4.5±0.4; FGRS 5.2±0.4; CON 5.6±0.4 $g \cdot kg^{-1} \cdot d^{-1}$; $p<0.05$ for FGRI vs CON) but similar weight, abdominal circumference and HT length. FGRS females had decreased CRL at 3 weeks and HT at 4 weeks compared with CON (both, $p<0.05$), but these differences were no longer statistically significant at weaning (Figure 1, A-D).

In males, FGRI lamb weight, CRL and abdominal circumference were less than CON initially, but by 4 weeks these measurements were similar amongst all three groups (Figure 1, E-G). FGRS and FGRI HT were less than CON at 4 weeks, but not thereafter (Figure 1H).

There were no differences between FGRI and FGRS in any of these parameters (Figure 1).

Post-weaning linear growth. In females, FGRI and FGRS sheep had reduced CRL compared with CON from 4 through 15 months, and at 18 months in FGRI (all, $p<0.05$) (Figure 2, B). Similarly, in FGRI and FGRS sheep HT from 5 through 12 months, and abdominal circumference from 5 through 7 months also were less than in CON (all, $p<0.05$) but weight was not significantly different at any time (Figure 2, A-D).

In males, weight was not different at any time and differences in linear growth were less marked, with HT length less in FGRI and FGRS than CON from 15 through 17 months, and least in FGRS ($p<0.05$) (Figure 2, E-H).

There were no differences in growth velocity for weight or linear growth amongst groups (data not shown).

Body composition. In females, lean mass was decreased in both FGRI and FGRS at 4 months of age compared with CON ($p<0.05$), and tended to be less in FGRS, but not FGRI, males ($p=0.06$). Bone mineral content also tended to be less in female ($p=0.07$), but not male, FGR compared with CON (Table 1).

There were no differences amongst groups in DXA measures at 12 months of age in either sex (Supplementary Table 2).

At 18 months of age in females FGRI had increased total lean mass relative to body weight ($p<0.05$) compared with CON, and increased rump lean mass compared with both CON and FGRS ($p=0.05$, $p<0.05$, respectively). However, abdominal lean mass in FGRI was less than CON and chest lean mass was decreased compared with both CON and FGRS ($p<0.05$). Accretion of lean mass between 4 and 18 months of age was greater in female FGRI and FGRS than in CON ($p<0.05$, Table 1).

FGRI females also had decreased abdominal fat mass relative to total fat mass than both CON and FGRS (p<0.05, Table 1). In males, body composition parameters at 18 months were not different amongst groups.

Post-mortem. Female FGRI sheep were lighter at PM than CON with lighter absolute and relative heart weight (relative to body surface area), lighter thyroid weight, but greater liver weight relative to live weight (all p<0.05) compared with CON. Female FGRS sheep had lighter cerebellae (p<0.05) compared to CON and lighter relative cerebellar weight compared with FGRI (p<0.05; Table 2). Male FGRS were lighter than CON with decreased carcass weight and lesser relative brain weight than CON (p<0.05; Table 2).

Pre-weaning glucose and insulin sensitivity. In females, FGRI lambs had a greater reduction in HOMA-determined β-cell function, but not in HOMA-determined insulin resistance, from 2 weeks to weaning compared with both CON and FGRS (Figure 3, A-D). In males, there were no differences amongst groups (Figure 3, E-H); however, increased weight at weaning in FGRI and FGRS male lambs was associated with reduced pre-weaning β-cell function (Figure 4). There was no such relationship in male CON or in any of the female groups.

Adult glucose and insulin sensitivity. In females, glucose and insulin response to an intravenous glucose tolerance test were not different amongst groups for any calculated measure (Table 3), although there was a significant time*experimental group interaction of insulin response over the entirety of the test, with CON having highest insulin concentrations (Figure 5). In the HGC, FGRS females had a 50%

reduction in mean glucose disposition compared with CON (p=0.06), required ~40% less glucose to maintain steady state compared with FGRI (p<0.05) with similar requirements to CON, but had no change in insulin secretion or insulin sensitivity. In males, FGRS sheep had poorer glucose tolerance compared to CON with increased peak plasma glucose during the IVGTT (Figure 5), increased peak change from baseline and decreased insulin secretion relative to glucose (all p<0.05; Table 3). There were no significant differences in males amongst groups during the HGC (Table 4).

Adult adrenergic sensitivity and lipid mobilisation. In females, there were no differences amongst groups in the first phase βHBA or NEFA responses to epinephrine but FGRI female sheep had an increased first phase Glucose:NEFA ratio (p<0.05) compared with CON and βHBA:NEFA ratio (p<0.05) compared with both FGRS and CON (Table 5). In males, FGRI had a blunted glucose response in response to epinephrine in the first 7.5 minutes compared with CON (p<0.05), but were not different from FGRS (Supplementary Figure 2; Table 5).

In both females and males, in FGRI abdominal fat mass was positively correlated with peak first phase NEFA response (female: p<0.0001, male: p<0.008). These associations were weaker, and not statistically significant, in female CON and FGRS and in male FGRS and absent in male CON (Figure 7).

Discussion

Fetal growth restriction remains a significant clinical challenge in diagnosis, management, and the long-term consequences for the affected baby. Whilst various intrauterine therapies have been investigated in pre-clinical animal studies (50),

progression to clinical trials is limited due to a lack of efficacy. We previously have shown that intra-amniotic IGF1 treatment of the growth-restricted ovine fetus improves fetal growth, restores gut development and up-regulates placental amino acid transporters (5, 17, 53). We have also reported that this therapy does not result in increased perinatal morbidity or mortality, a potential concern when growth is improved in an environment of relative deprivation (51). However, IGF1 is a growth-promoting hormone and, therefore, long-term assessment of safety and efficacy is necessary. In this study we demonstrate that fetal treatment of growth restriction with low doses of intra-amniotic IGF1 improves body composition and alters metabolic flexibility in adulthood in a sexually dimorphic manner.

Growth-restricted lambs caught up with respect to weight, but not linear growth, by 4 months of age (post-weaning). Linear growth, as assessed by crown-rump length and hock-toe length, was similar to control animals by young adulthood. However, in both FGRI and FGRS males, greater weight at weaning was strongly correlated with a greater reduction in basal insulin secretion in the pre-weaning period. This suggests increased insulin sensitivity in growth-restricted offspring during the pre-weaning period, and is consistent with other paradigms of FGR in sheep (13, 39). This relationship was not evident in female lambs, but FGRI females had a greater reduction in basal insulin secretion compared to both FGRS and CON, which could suggest a compensatory adaptation to greater insulin sensitivity across the pre-weaning period. The decreased acute insulin response, and greater requirement for glucose to maintain steady state during the hyperglycaemic clamp in adult female FGRI sheep compared with CON, could also suggest improved insulin sensitivity in IGF1-treated adult females. These persistent changes indicate that fetal IGF1 therapy beneficially influences metabolic health in the growth-restricted adult.

Previous observations in SGA children suggest that decreased pancreatic β-cell compensation is associated with increased propensity for visceral adipose deposition (35). We did not find increased abdominal adiposity in FGRS females compared with CON, despite a trend towards reduced glucose disposition and reduced lean mass; however, IGF1 therapy altered lean and fat mass compartmentalization in females, in particular reducing abdominal adipose. Abdominal obesity is associated with metabolic syndrome and cardiovascular disease risk (14). The selective reduction in abdominal adipose in female offspring has clinical implications in the long-term maintenance of cardiometabolic health in FGR offspring. In male FGRS sheep, the impaired glucose tolerance compared with CON despite a greater insulin response, and reduced insulin sensitivity compared to both CON and FGRI suggest that FGR in the male is associated with decreased peripheral sensitivity of glucose uptake to insulin, possibly due to reduced skeletal muscle insulin sensitivity (12). Interestingly, both live and empty carcass weight in FGRS males were significantly less than CON at post-mortem suggest a failure to catch-up, which is consistent with a recent meta-analysis of human datasets demonstrating that low birth weight individuals may have a decreased risk of being overweight (47). Intra-amniotic IGF1 Intervention in males resulted in greater capacity for L-Arginine-stimulated insulin secretion, which approaches significance when compared with FGRS. These data are compatible with the increased pancreatic insulin sequestration observed in the studies of Limesand and colleagues (33). Although a greater than 50% increase in insulin sensitivity in FGRI males compared with FGRS was not significant at this relatively young age, future studies should investigate whether manifest dysfunction in homeostatic control of the glucose-insulin axis due to FGR is corrected by intra-amniotic IGF1 treatment at a more advanced age.

Of significant interest are the sex-specific effects of this treatment on both metabolic regulation and body composition. Lesser abdominal adipose accumulation and a striking increase in lean mass from the pre- to post-pubertal period in females could suggest that the complex gonadotrophic and somatotrophic interactions which occur during puberty (52) may be altered by intra-amniotic IGF1 intervention. Increased lean tissue accretion could be due to greater skeletal muscle somatotrophic sensitivity, augmented by peripheral nutrient signalling pathways and glucose utilisation. Additionally, greater adrenergic sensitivity in FGRI-treated offspring could indicate a shift in substrate mobilization during periods of stress. These data suggest that intra-amniotic IGF1 intervention of the growth-restricted fetus recovers growth restriction-mediated adrenergic desensitisation (8), and have significant implications for the maintenance of age-appropriate body composition and metabolic regulation in FGR adults at risk for the development of NCDs.

Interestingly, FGRI sheep of both sexes show a strong positive correlation between abdominal fat mass at 18 months and peak adrenaline-stimulated plasma NEFA concentration, suggesting that mobilisation is appropriate to abdominal substrate sequestration. Unfortunately, from the current data we cannot determine the primary origin of adrenergic-stimulated lipolysis or glycogenolysis. Molecular analyses of adrenergic receptor expression and tissue-specific glycolytic, lipolytic, and oxidative capacity are required to determine whether intra-amniotic IGF-1 treatment contributes to substrate selection and metabolic capacity, which is impaired in the growth-restricted lamb (8, 54). If abnormal depot-specific tissue accretion, which is evident in the liver of FGR and SGA humans (1, 18), is corrected with intra-amniotic IGF1-intervention, this could be associated with the recovery of an appropriate metabolic phenotype.

Perspectives and Significance

We report significant improvement in adult metabolic function and body composition after intra-amniotic treatment of FGR. This is the first study to demonstrate persistent, beneficial, long-term effects of a prenatal intervention. However, metabolic testing in young adulthood may be too early to identify long-term effects of the intervention and physiological significance. Indeed, as insulin sensitivity and secretion wane with ageing, studies in later life would help determine whether these findings become more apparent with age and, therefore, the potential of this prenatal intervention to preventing chronic diseases of ageing should the findings translate to the human. Our study extends previous knowledge about sexually dimorphic adaptations to FGR and intra-amniotic IGF1 intervention in the newborn period by showing that these persist and develop through 18 months of age in the placental insufficiency-induced growth-restricted sheep. Further work is needed to elucidate whether these sex-specific responses are due to an improved prenatal environment, or compensatory adaptations to the intervention in later life.

The burgeoning increase in NCDs such as metabolic syndrome, diabetes, cardiovascular diseases, and obesity, indicate that current preventative interventions are inadequate. However, this work provides proof-of-principle that a brief fetal intervention has long-term sex-specific effects in fetal growth restricted offspring. We demonstrate that early-life intervention, before the progression to symptomatic metabolic dysfunction in early adulthood, improves clinically relevant aspects of growth and metabolism.

Author Contributions

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conceived and designed experiments: FHB, JEH, MHO, AMS

Acquired, analysed and interpreted data: AMS, FHB, MHO, TDG, ALJ

Drafted manuscript: AMS

Revised the manuscript, contributed to discussion and approved the final version: all authors

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Figure Legends

Figure 1. **Postnatal growth to weaning.** Weight, crown rump length (CRL), abdominal circumference, and hock-to-toe (HT) length in female (A-D) CON (white, *n*=14), FGRS (grey, *n*=15), FGRI (black, *n*=13), and male (E-H) CON (white, *n*=22), FGRS (grey, *n*=12), FGRI (black, *n*=18) lambs from 2-12 weeks of age. Data are mean

464 ± SEM. Symbols denote *p*-values for differences between experimental groups at each
465 time point (ANOVA: ***** FGRS vs. CON, $p<0.05$; † FGRI vs. CON, $p<0.05$).
466
467 ***Figure 2.*** **Postnatal growth from weaning to adulthood.** Weight, crown rump length
468 (CRL), abdominal circumference, and hock-to-toe (HT) length in female (A-D) CON
469 (white, *n*=12), FGRS (grey, *n*=14), FGRI (black, *n*=13), and male (E-H) CON (white,
470 *n*=21), FGRS (grey, *n*=10), FGRI (black, *n*=12) sheep from 4 to 18 months of age.
471 Data are mean ± SEM. Symbols denote p-values for differences between experimental
472 groups at each time point (ANOVA: ***** FGRS vs. CON, $p<0.05$; † FGRI vs. CON,
473 $p<0.05$; ‡ FGRI vs. FGRS, $p<0.05$).
474
475 ***Figure 3.*** **Postnatal Homeostasis Model of Assessment through weaning.**
476 Homeostasis Model of Assessment of β-cell function (HOMA%-β) and insulin
477 sensitivity (HOMA-%S) from 2-12 weeks of age, and the change in HOMA-β and
478 HOMA-S from 2-12 weeks in: (A-D) female CON (white, *n*=12), FGRS (grey, n=9) and
479 FGRI (black, *n*=12), and (E-H) male CON (white, *n*=15), FGRS (grey, *n*=11) and FGRI
480 (black, *n*=9) lambs. Data are mean ± SEM. Symbols denote p-values for differences
481 between experimental groups at each time point (ANOVA: † FGRI vs. CON, $p<0.05$;
482 ‡ FGRI vs. FGRS, $p<0.05$).
483
484 ***Figure 4.*** **The change in β-cell function through weaning relative to weaning**
485 **weight in males.** The correlation between the change in Homeostatic Model of
486 Assessment of β-cell function (HOMA-%β) from 2-12 weeks of age, and weight at
487 weaning (12 weeks) in male CON (white and dotted line, *n*=15), FGRS (grey, *n*=11)
488 and FGRI (black, *n*=9) lambs. Significant correlations are indicated.

Figure 5. **Glucose tolerance in adulthood.** Plasma glucose and insulin response to an intravenous glucose tolerance test in female (A, B) CON (white, *n*=7), FGRS (grey, *n*=10) and FGRI (black, *n*=9) and male (C, D) CON (white, *n*=9), FGRS (grey, *n*=9) and FGRI (black, *n*=8) sheep. Data are mean ± SEM. Roman numerals denote the significant difference between experimental groups (RM ANOVA: *iii*, time*experimental group interaction $p<0.05$). SEM. Symbols denote *p*-values for differences between experimental groups at each time point (ANOVA: ***** CON vs. FGRS, $p<0.05$).

Figure 6. **Glucose-stimulated insulin regulation in adulthood.** Plasma glucose (circles) and insulin (squares) response to an intravenous hyperglycemic clamp with period of steady state hyperglycemia (SS) and following an L-arginine bolus (ARG) in female (A) CON (white, *n*=8), FGRS (grey, *n*=9) and FGRI (black, *n*=9) and male (B) CON (white, *n*=8), FGRS (grey, *n*=7) and FGRI (black, *n*=7) sheep. Data are mean ± SEM. Roman numerals denote the significant difference between experimental groups (RM ANOVA: *i*, main effect of experimental group, $p<0.05$; *ii*, effect of time, $p<0.05$; *iii*, time*experimental group interaction, $p<0.05$). Symbols denote p-values for differences between experimental groups at each time point (ANOVA: ‡ FGRS vs. FGRI, $p<0.05$).

Figure 7. **Correlation between peak plasma non-esterified fatty acid response to epinephrine and abdominal fat.** The correlation between peak plasma non-esterified fatty acid (NEFA) concentration following an epinephrine stimulation test and abdominal fat by dual X-ray absorptiometry in female (A) CON (white and dotted line,

513 *n*=9), FGRS (grey, *n*=11) and FGRI (black, *n*=10), and male (B) CON (white and dotted
514 line, *n*=9), FGRS (grey, *n*=7) and FGRI (black, *n*=8) lambs.

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Figures and Tables
Figure 1

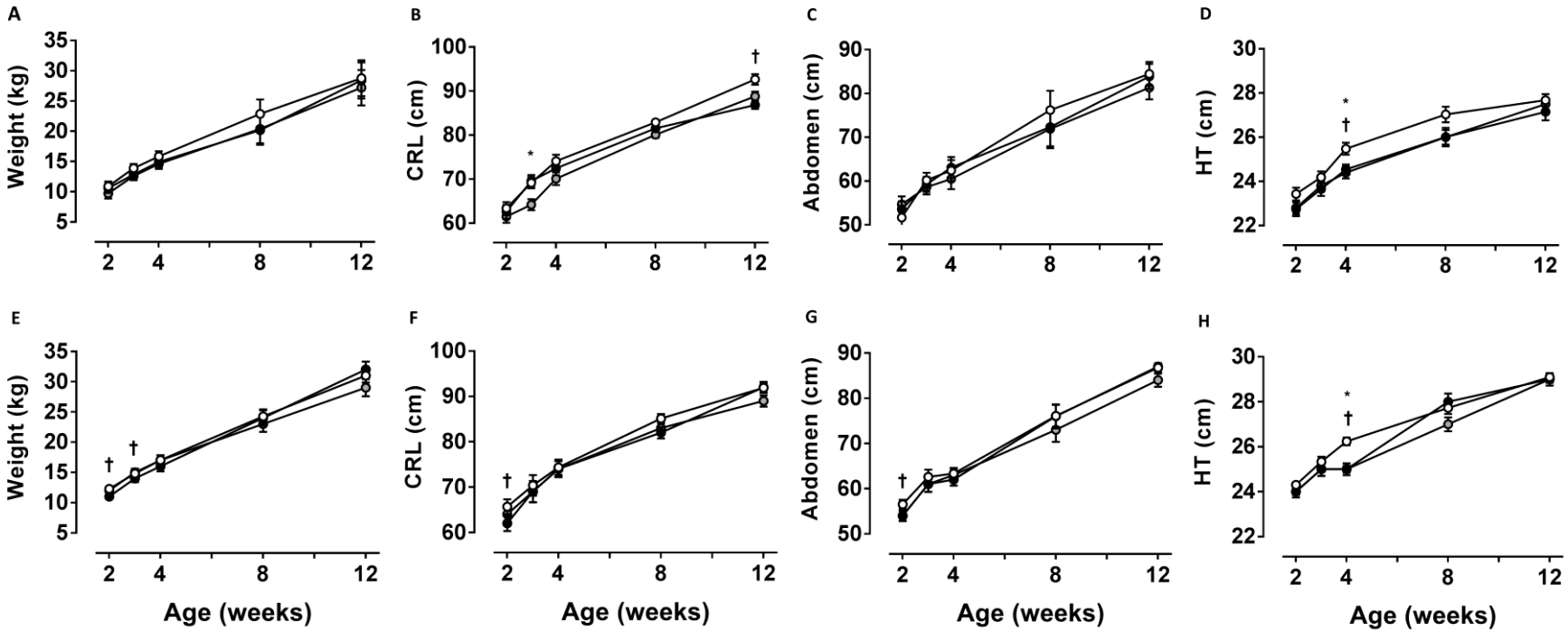
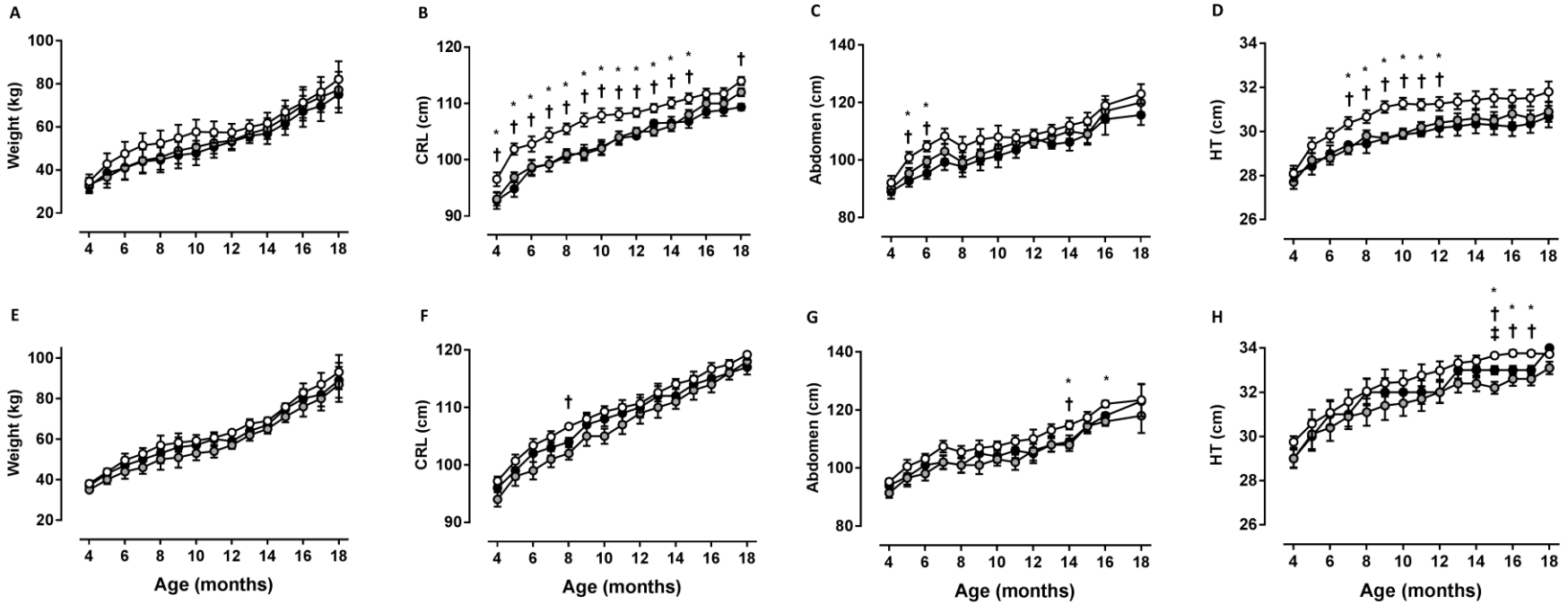


Figure 2



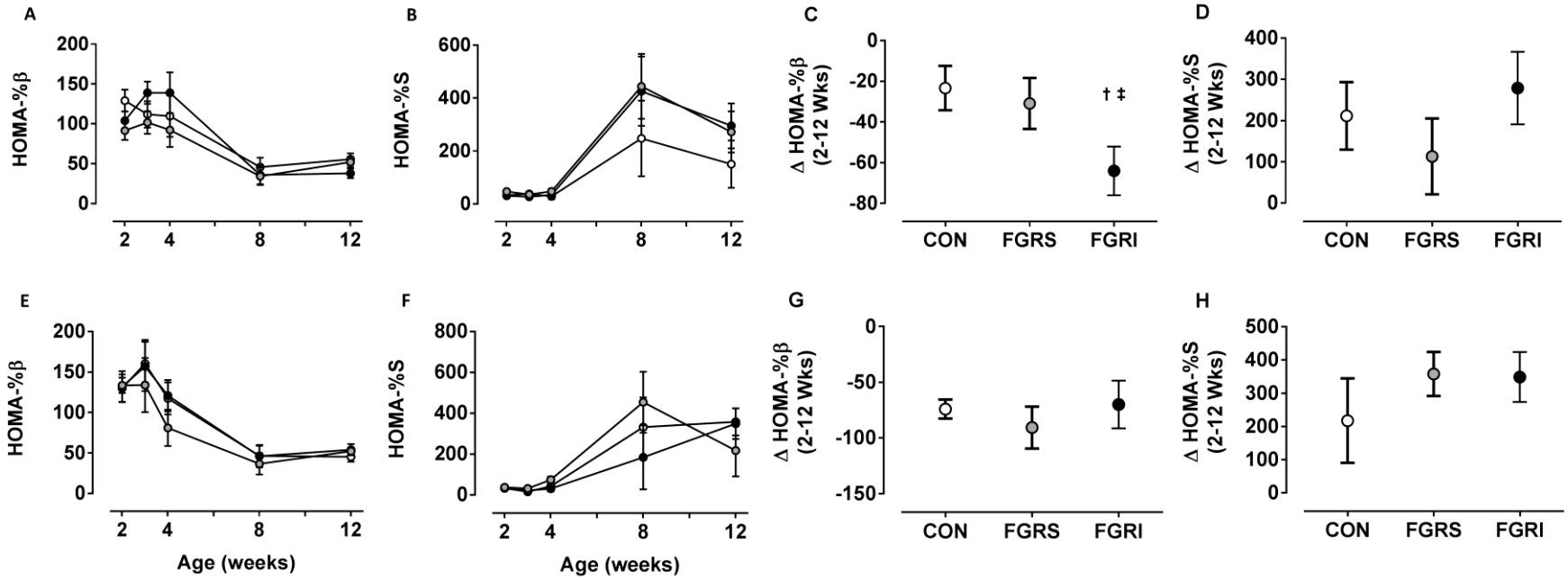
	Female			Male			Significance (treatment effect)	
4 months	CON *n*=12	FGRS *n*=15	FGRI *n*=13	CON *n*=21	FGRS *n*=11	FGRI *n*=16	Female	Male
BMC (g)	402±41	353±41	360±42	384±11	374±18	392±20	0.07*	ns
BMD (g·cm^{-2})	0.99±0.05	0.91±0.04	0.94±0.05	0.91±0.03	0.95±0.04	0.95±0.04	ns	ns
LM (kg)	28.6±2.0	25.8±2.0	25.3±2.0	31.4±1.0	28.9±1.2	29.9±1.1	<0.05*†	0.06*
LM:WT (%)	79.6±2.9	77.9±2.9	77.8±2.9	82.2±0.9	82.1±1.2	80.5±1.2	ns	ns
18 months	CON *n*=12	FGRS *n*=13	FGRI *n*=12	CON *n*=21	FGRS *n*=10	FGRI *n*=13		
BMC (kg)	1.3±0.2	1.2±0.2	1.1±0.2	1.3±0.1	1.2±0.1	1.4±0.1	ns	0.08‡
Chest LM (kg)	15.0±0.3	15.2±0.3	13.5±0.4	20.8±1.0	19.2±1.1	20.6±1.0	<0.05†‡	ns
Chest FM (kg)	6.1±1.7	5.3±1.7	4.6±1.7	3.3±0.9	2.7±1.0	3.1±1.0	ns	0.07*
Abdominal LM (kg)	24.7±0.5	24.1±0.5	22.2±0.9	31.1±1.9	30.1±2.0	29.4±2.0	0.05†	ns
Abdominal FM (kg)	10.0±2.8	8.5±2.8	7.9±2.8	5.1±1.5	4.3±1.6	4.5±1.6	ns	ns
Rump LM (kg)	10.5±1.1	10.4±1.2	16.1±1.6	13.9±0.6	15.7±0.9	13.4±1.1	0.05†, <0.05‡	ns
Rump FM (kg)	4.2±1.0	4.4±1.0	5.0±1.0	2.5±0.4	2.0±0.4	2.4±0.4	ns	ns
Total LM (%)	50.4±1.3	50.1±1.3	52.8±1.0	67.6±1.6	65.5±2.0	64.3±2.0	ns	ns
Total FM (%)	20.3±5.9	18.2±5.9	18.7±5.9	11.0±3.0	9.0±3.0	9.7±3.0	ns	ns
LM:WT (%)	61.4±8.2	67.6±8.2	70.6±8.2	71.1±2.8	73.0±3.0	71.0±3.0	<0.05†	ns
FM:WT (%)	24.1±5.0	23.1±4.9	23.8±5.1	11.8±2.1	9.9±2.2	11.2±2.2	ns	ns
Abdominal FM:WT (%)	11.9±2.4	10.8±2.4	10.0±2.4	5.4±1.2	4.7±1.3	4.9±1.3	ns	ns
Abdominal FM:Total FM (%)	49.3±0.9	48.5±1.0	43.7±1.2	46.0±2.0	46.1±2.2	45.8±2.1	<0.05†‡	ns
$GV_{4\text{-}18}$ LM (g·kg^{-1}·d^{-1})	1.4±0.3	1.8±0.3	1.8±0.3	1.9±0.1	2.0±0.1	2.1±0.1	<0.05*†	ns

Table 1. **Body composition at 4 and 18 months of age.** Bone mineral content (BMC), compartmental and total lean mass (LM) and fat mass (FM), absolute and, where appropriate, relative to weight (WT); growth velocity of lean mass from 4-18 months of age ($GV_{4\text{-}18}$ LM). Data are mean ± SEM. Symbols denote differences amongst experimental groups on post hoc testing: * FGRS vs. CON; † FGRI vs. CON; ‡ FGRI vs. FGRS.

	Female			Male			Significance (treatment effect)	
	CON *n*=12	FGRS *n*=13	FGRI *n*=12	CON *n*=21	FGRS *n*=10	FGRI *n*=12	Female	Male
Live Weight (kg)	83.8±6.9	78.8±6.9	76.7±6.9	93.5±6.1	86.8±6.3	90.7±6.3	<0.05†	<0.05*
Carcass Weight (kg)	54.7±4.0	52.9±4.0	49.8±4.0	60.1±2.6	54.9±2.8	59.5±2.8	ns	<0.05*
Fat Depth, 12th Rib (mm)	22.9±3.4	21.0±3.3	19.0±3.4	16.3±2.9	14.5±3.1	12.8±3.0	ns	ns
Adrenals (g)	5.3±0.3	4.8±0.3	5.7±0.3	5.6±0.2	5.2±0.3	5.2±0.2	ns	ns
Brain (g)	106±3	102±3	102±3	110±2	115±2	111±2	ns	ns
Cerebellum (g)	11.8±0.5	10.9±0.5	11.6±0.5	13.1±0.1	13.0±0.3	13.0±0.2	<0.05*	ns
Heart (g)	329±16	305±16	299±16	377±16	368±19	354±18	<0.05†	ns
Kidneys (g)	230±10	209±10	219±11	279±11	280±13	292±13	ns	ns
Liver (g)	1,352±80	1,310±80	1,368±82	1,623±112	1,578±121	1,601±118	ns	ns
Lungs (g)	628±49	617±48	617±49	753±21	689±27	646±26	ns	ns
Pancreas (g)	97±7	81±6	86±7	103±4	101±5	113±5	ns	ns
Pancreatic Lymph Node (g)	1.6±0.3	2.2±0.3	2.3±0.3	2.7±0.2	2.1±0.3	1.8±0.3	ns	<0.05†
Perirenal Fat (g)	1,061±152	1,072±150	932±154	505±73	442±79	419±78	ns	ns
Thymus, neck (g)	32.9±7.0	30.6±6.6	35.5±6.9	20.5±4.8	23.2±5.5	23.5±5.3	ns	ns
Thyroid (g)	10.6±1.3	9.1±1.3	7.2±1.4	9.4±1.6	9.1±1.6	7.7±1.6	<0.05†	ns
Brain:WT (%)	0.13±0.01	0.13±0.01	0.14±0.01	0.12±0.01	0.13±0.01	0.12±0.01	ns	<0.05*
Cerebellum:WT (%)	0.014±0.001	0.014±0.001	0.015±0.001	0.014±0.001	0.015±0.001	0.015±0.001	<0.05‡	ns
Heart WT:BSA ($g \cdot m^{-2}$)	2,719±123	2,529±121	2,486±123	3,084±109	3,031±133	2,943±132	<0.05†	ns
Kidney:WT (%)	0.27±0.01	0.27±0.01	0.29±0.01	0.30±0.01	0.30±0.01	0.31±0.01	ns	ns
Liver:WT (%)	1.62±0.06	1.67±0.06	1.81±0.06	1.73±0.02	1.86±0.05	1.78±0.03	<0.05†	ns
Pancreas:WT (%)	0.12±0.01	0.10±0.01	0.12±0.01	0.11±0.01	0.12±0.01	0.12±0.01	ns	ns
BMI ($kg \cdot m^{-2}$)	63.3±6.5	61.8±6.5	62.1±6.5	65.8±6.6	65.2±6.7	64.2±6.7	ns	ns

Table 2. **Post mortem carcass and organ weights and measures.** Live weight (WT), body surface area (BSA), body mass index (BMI). Data are mean ± SEM. Symbols denote differences amongst experimental groups on post hoc testing: * FGRS vs. CON; † FGRI vs. CON; ‡ FGRI vs. FGRS.

Figure 3


A
HOMA-%β
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150
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Δ HOMA-%β (2-12 Wks)
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-20
-40
-60
-80
† ‡
CON
FGRS
FGRI
D
Δ HOMA-%S (2-12 Wks)
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Age (weeks)

Figure 4

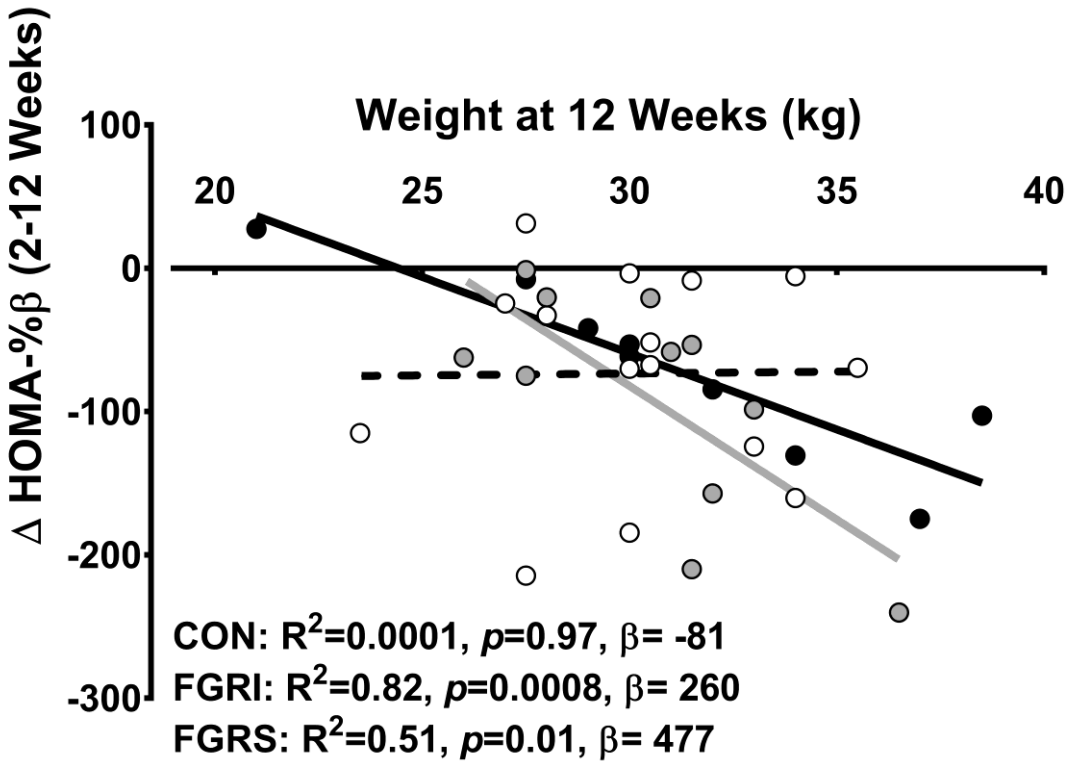

Weight at 12 Weeks (kg)
20
25
30
35
40
100
0
-100
-200
-300
Δ HOMA-%β (2-12 Weeks)
CON: R2=0.0001, p=0.97, β= -81
FGRI: R2=0.82, p=0.0008, β= 260
FGRS: R2=0.51, p=0.01, β= 477

Figure 5

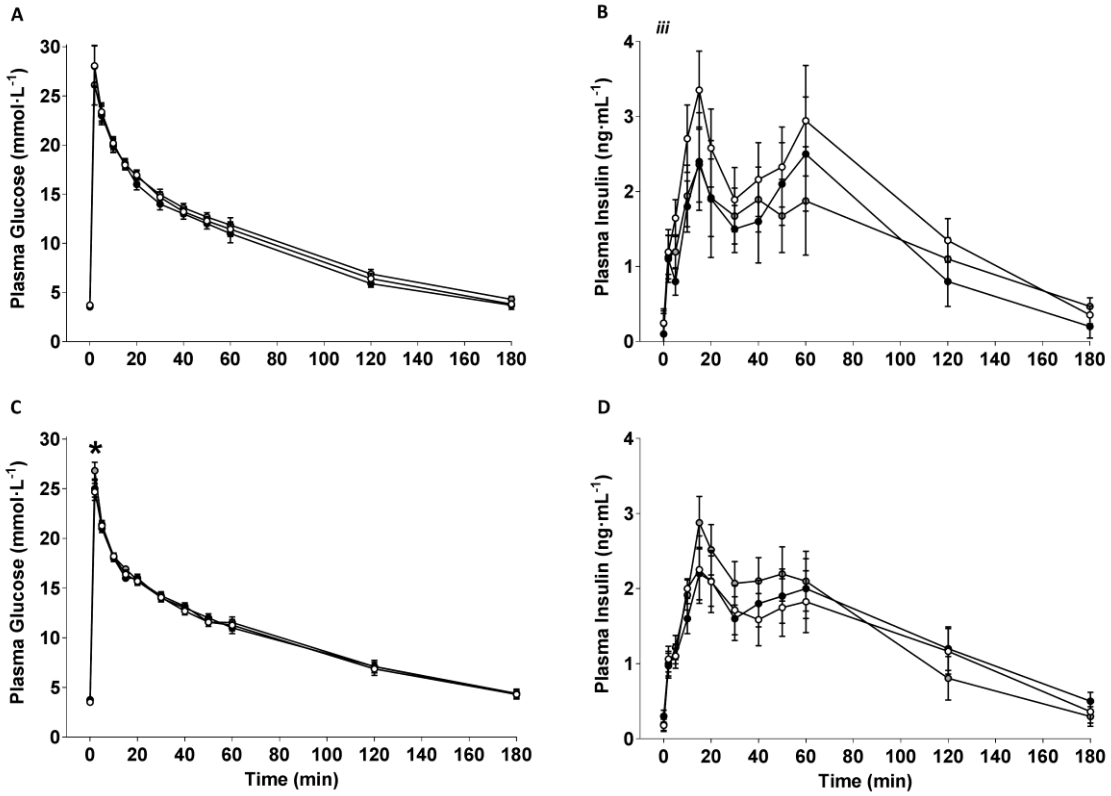
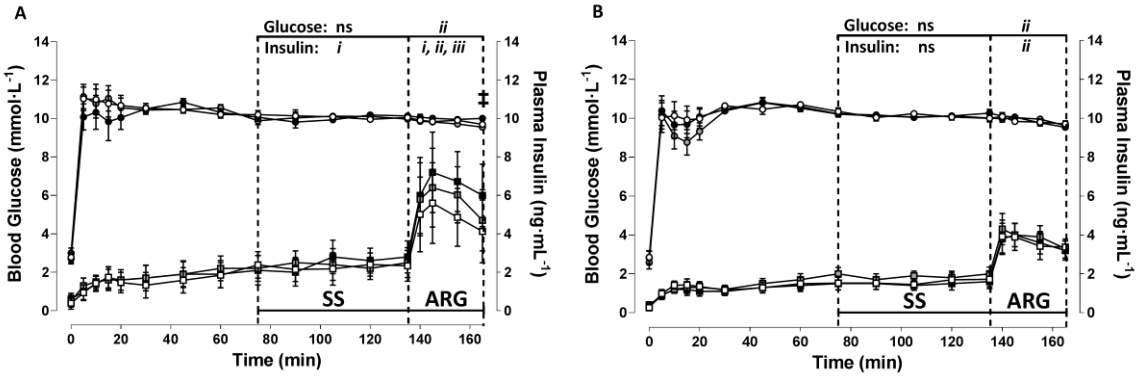


Figure 6



	Female			Male			Significance (treatment effect)	
	CON *n*=10	FGRS *n*=10	FGRI *n*=10	CON *n*=9	FGRS *n*=8	FGRI *n*=8	Female	Male
IVGTT-AUC$_{G1}$ (mmol·L^{-1})	192±10	191±10	186±10	169±4	174±4	169±4	ns	ns
IVGTT-AUC$_{G2}$ (mmol·L^{-1})	855±71	891±65	783±52	894±67	945±67	840±64	ns	ns
Peak Δ Glucose (mmol·L^{-1})	24.3±1.9	23.8±1.9	23.9±1.9	21.1±0.8	23.1±0.8	21.7±0.8	ns	<0.05*
IVGTT-AUC$_{I1}$ (ng·mL^{-1})	27.4±4.1	18.6±4.1	17.0±6.4	17.6±2.9	19.6±2.0	16.5±2.0	ns	ns
IVGTT-AUC$_{I2}$ (ng·mL^{-1})	218±48	175±44	139±59	196±42	218±41	189±41	ns	ns
Peak Δ Insulin (ng·mL^{-1})	2.8±0.7	2.4±0.6	2.9±0.8	2.3±0.4	3.0±0.3	2.2±0.3	ns	ns
Δ I_{S1} (ng·mL^{-1})	2.7±0.5	2.2±0.4	2.2±0.4	2.1±0.2	2.8±0.3	1.9±0.2	ns	ns
AIR$_{15}$ (ng·mL^{-1})	17.5±3.6	15.4±3.1	13.6±4.0	16.3±1.3	16.7±1.9	12.3±1.9	ns	ns
ln I_S (ng·mL^{-1}·mmol·L^{-1})	-1.7±0.4	-1.8±0.4	-1.8±0.4	-1.8±0.2	-1.6±0.2	-1.7±0.2	ns	<0.05*

Table 3. **Glucose tolerance in adulthood.** Intravenous glucose tolerance test (IVGTT) first (AUC-G_1) and second (AUC-G_2) phase glucose response, peak glucose change (Δ), first (AUC-I_1) and second AUC-I_2) phase insulin response, peak change (Δ) in insulin secretion, first phase insulin response change from baseline (Δ I_{S1}), insulin response (AIR_{15}), and insulin secretion (I_S) throughout the test. Data are mean ± SEM. Symbols denote significant differences amongst experimental groups upon post hoc testing: * CON vs. FGRS.

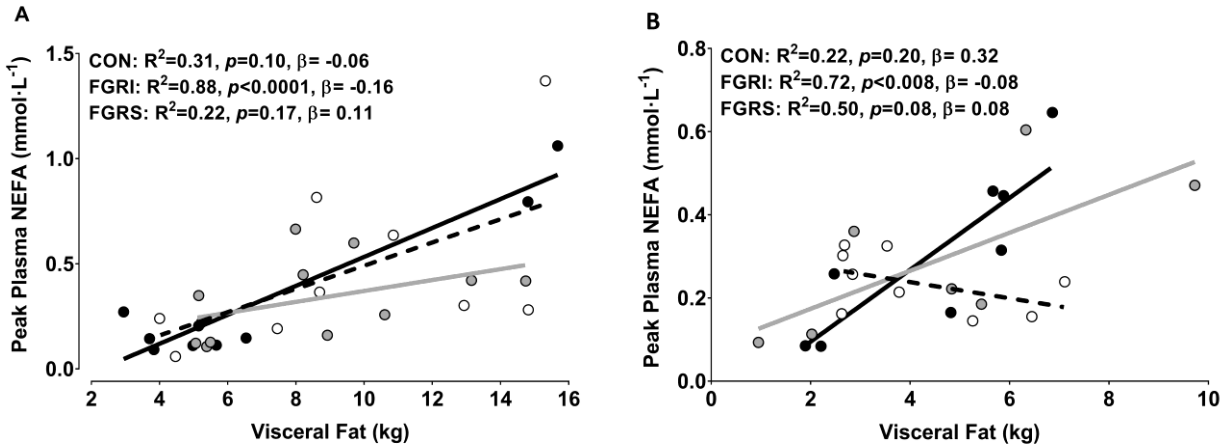
	Female			Male			Significance (treatment effect)	
	CON *n*=9	FGRS *n*=10	FGRI *n*=10	CON *n*=9	FGRS *n*=8	FGRI *n*=8	Female	Male
HGC-AUC$_{ISS}$ (ng·mL^{-1})	109±48	114±46	163±48	75±7	93±11	64±11	ns	ns
HGC-AUC$_{IARG}$ (ng·mL^{-1})	115±29	134±26	159±26	91±8	93±12	79±12	ns	ns
HGC-AUC$_{GSS}$ (mmol·L^{-1})	596±6	594±6	592±6	599±3	602±3	598±3	ns	ns
HGC-AUC$_{GARG}$ (mmol·L^{-1})	293±3	290±3	300±3	292±2	293±2	295±2	<0.05‡	ns
IG$_{SS}$ (mg·kg^{-1}·min^{-1})	7.6±0.9	6.0±0.9	9.6±1.0	7.0±0.9	7.2±0.6	6.4±0.6	<0.05‡	ns
IG$_{ARG}$ (mg·kg^{-1}·min^{-1})	8.4±1.3	8.3±1.3	10.4±1.3	8.2±0.3	9.1±0.6	6.7±0.6	ns	ns
Mean I$_{SS}$ (ng·mL^{-1})	2.3±1.1	2.5±1.1	3.3±1.1	1.6±0.3	1.9±0.3	1.5±0.3	ns	ns
Peak I$_{ARG}$ (ng·mL^{-1})	5.7±2.7	6.9±2.6	8.4±2.7	4.4±0.8	4.5±0.8	4.3±0.8	ns	ns
ΔI$_S$ ARG:SS	2.7±0.2	2.9±0.2	2.6±0.1	2.7±0.1	2.3±0.2	2.8±0.2	ns	ns
S$_i$ SS (mg·kg^{-1}·min^{-1}·ng·mL^{-1})	4.5±1.3	3.4±1.2	5.0±1.3	4.3±0.7	3.5±0.8	5.4±1.3	ns	ns
S$_i$ ARG (mg·kg^{-1}·min^{-1}·ng·mL^{-1})	2.5±0.7	1.8±0.7	2.8±0.7	2.5±0.4	2.5±0.4	2.7±0.4	ns	ns
DI$_G$ (mg·kg^{-1})	70.9±12.5	35.9±11.5	64.5±12.4	58.2±15.4	60.6±15.2	75.5±15.2	0.06*	ns
GSIS (ng·mL^{-1})	1.9±0.6	1.9±0.6	2.2±0.6	1.3±0.3	1.6±0.2	1.1±0.2	ns	ns

Table 4. **Glucose-mediated insulin response in adulthood.** Absolute insulin and glucose responses to a hyperglycemic clamp (HGC): Area under the curve (AUC) for plasma insulin (I) and glucose (G) during steady state (HGC-AUC$_{ISS}$, HGC-AUC$_{GSS}$) and in response to L-Arginine bolus (HGC-AUC$_{IARG}$, HGC-AUG$_{IARG}$), glucose infusion rate (IG) (mmol·kg·min^{-1}) during steady state (IG$_{SS}$) and in response to an L-Arginine bolus (IG$_{ARG}$), mean plasma insulin concentration (ng·mL^{-1}) during steady state (I$_{SS}$) and peak plasma insulin concentration in response to an L -Arginine bolus (I$_{ARG}$), change in insulin secretion from steady state to the peak following the L-Arginine bolus (ΔI$_S$), insulin sensitivity (S$_i$), glucose disposition index (DI$_G$), and glucose-stimulated insulin secretion (GSIS). Data are mean ± SEM. Symbols denote significant differences amongst experimental groups upon post hoc testing: * FGRS vs CON; † FGRI vs. CON; ‡ FGRI vs. FGRS.

	Female			Male			Significance (treatment effect)	
	CON *n*=9	FGRS *n*=10	FGRI *n*=10	CON *n*=9	FGRS *n*=8	FGRI *n*=8	Female	Male
EPI-AUC$_{G7.5}$ (mmol·L^{-1})	5.6±0.5	5.7±0.5	6.2±0.4	6.0±1.0	5.3±1.0	4.0±1.1	ns	<0.05†
EPI-AUC$_{GT}$ (mmol·L^{-1})	44.5±5.6	45.5±4.9	37.9±5.2	36.1±4.6	40.1±5.2	34.1±4.6	ns	ns
ln EPI-AUC $_{βHBA7.5}$ (mmol·L^{-1})	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.2	0.3±0.1	0.5±0.1	ns	ns
ln EPI-AUC$_{βHBAT}$ (mmol·L^{-1})	0.8±0.4	0.4±0.3	1.3±0.4	0.8±0.3	1.1±0.3	0.6±0.3	ns	ns
ln EPI-AUC$_{NEFA7.5}$ (mmol·L^{-1})	1.1±0.3	1.1±0.2	0.7±0.3	1.1±0.2	1.1±0.2	0.9±0.2	ns	ns
ln EPI-AUC$_{NEFAT}$ (mmol·L^{-1})	2.2±0.8	2.1±0.8	1.1±0.8	1.5±0.5	1.5±0.5	1.6±0.5	ns	ns
Glucose:NEFA$_{7.5}$	16.5±12.9	25.3±12.6	40.4±12.8	23.9±12.9	34.5±12.8	37.3±12.8	<0.05†	ns
βHBA:NEFA$_{7.5}$	1.1±1.4	1.8±1.4	3.9±1.4	1.5±1.1	2.8±1.1	3.0±1.1	<0.05†‡	ns

Table 5. **Adrenergic sensitivity.** Absolute and natural log transformation (ln) of metabolite responses to epinephrine (EPI) stimulation: Area under the curve (AUC) for plasma glucose (G), β-hydroxybutyric acid (βHBA), and non-esterified fatty acids (NEFA) in the first 7.5 minutes (EPI-AUC$_{G7.5}$, EPI-AUC$_{NEFA7.5}$, and EPI-AUC$_{βHBA7.5}$) and 60 minutes (EPI-AUC$_{GT}$, EPI-AUC$_{NEFAT}$, and EPI-AUC$_{βHBAT}$), and peak glucose and βHBA relative to NEFA response at 7.5 minutes. Data are mean ± SEM. Symbols denote significant differences amongst experimental groups upon post hoc testing: † FGRI vs. CON; ‡ FGRI vs. FGRS.

Figure 7


A
CON: R2=0.31, p=0.10, β= -0.06
FGRI: R2=0.88, p<0.0001, β= -0.16
FGRS: R2=0.22, p=0.17, β= 0.11
Peak Plasma NEFA (mmol·L-1)
Visceral Fat (kg)
B
CON: R2=0.22, p=0.20, β= 0.32
FGRI: R2=0.72, p<0.008, β= -0.08
FGRS: R2=0.50, p=0.08, β= 0.08
Peak Plasma NEFA (mmol·L-1)
Visceral Fat (kg)

Effects of intrauterine insulin-like growth factor-1 therapy for fetal growth restriction on adult metabolism and body composition are sex-specific

Supplementary material

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Running title: Adult effects of fetal IGF1 therapy for FGR

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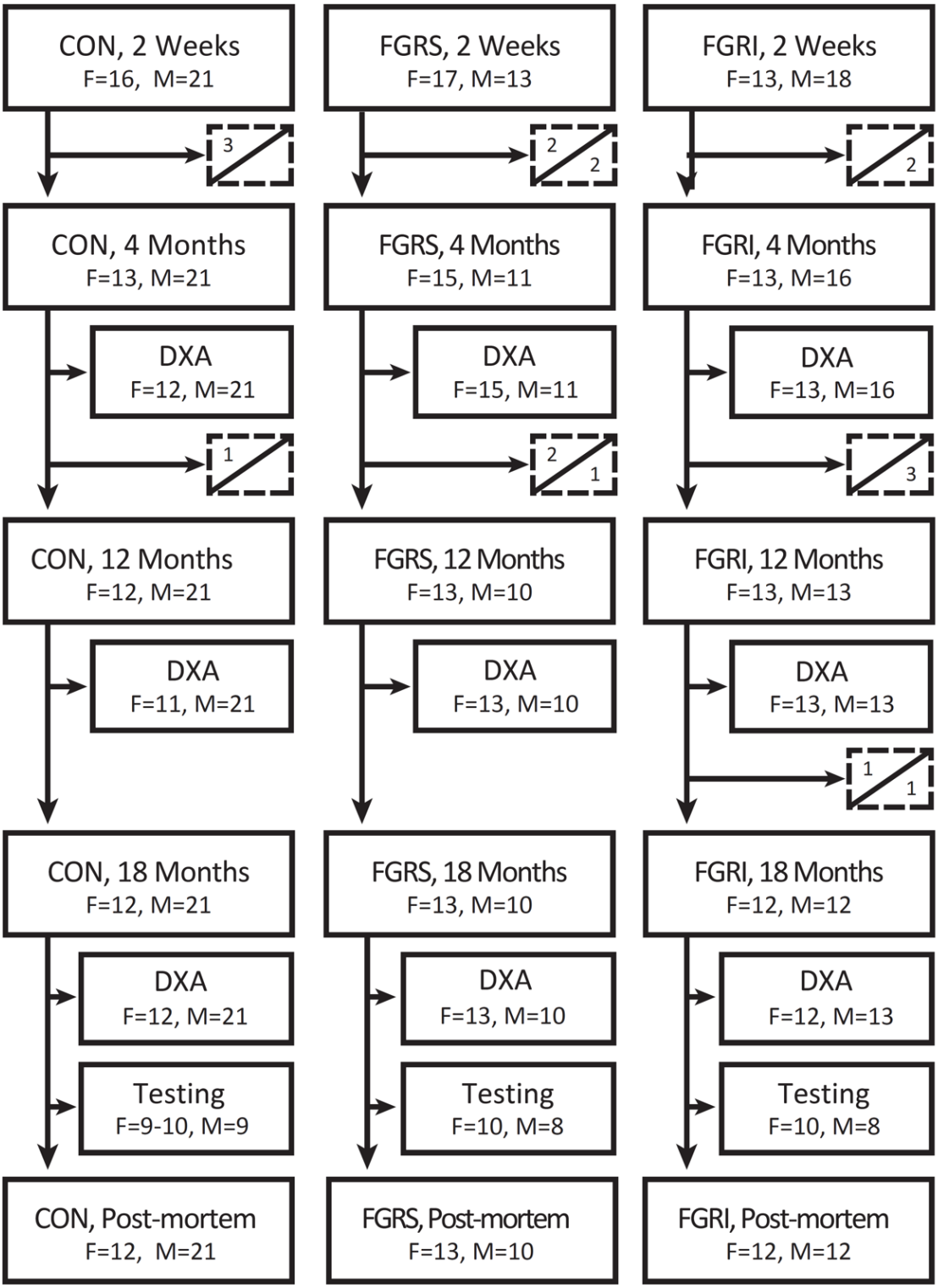
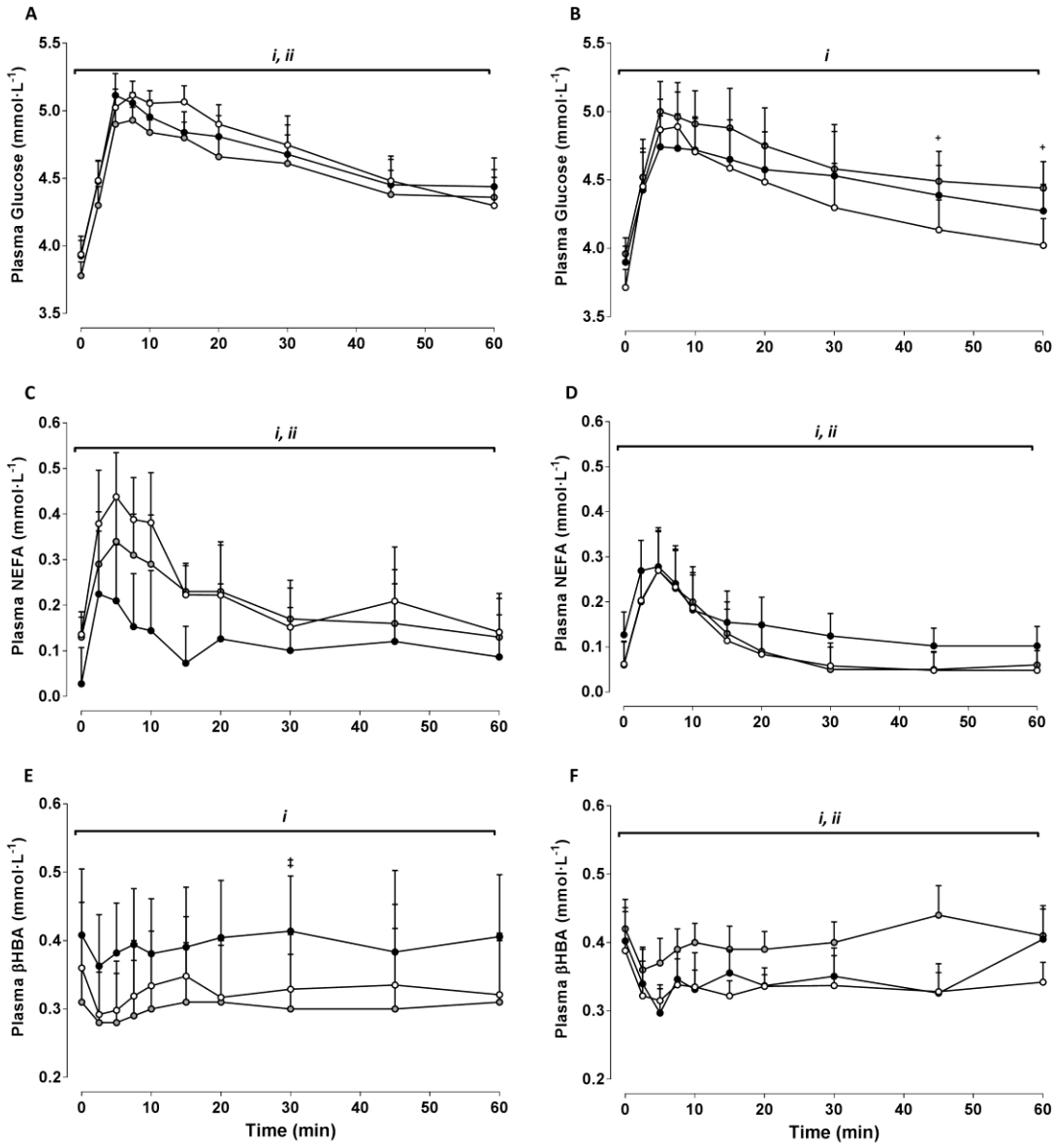


Supplementary Figure 1. **Animals utilised during the project according to experimental group and sex.** Dashed boxes report exclusions, deaths and euthanasia. Split boxes reporting postnatal deaths indicate the number of females (top left) and males (bottom right) for each experimental group. Circumstances constituting exclusion from the project and/or euthanasia were due to postnatal illness or paddock deaths.



Supplementary Figure 2. **Plasma metabolite response to intravenous epinephrine injection.** Plasma glucose, non-esterified fatty acids (NEFA), and β-hydroxybutyric acid (βHBA) response to an intravenous epinephrine injection in female (A, C, E: CON, white, *n*=9; FGRS, grey, *n*=10; FGRI, black, *n*=10) and male (B, D, F: CON, white, *n*=9; FGRS, grey, *n*=9; FGRI, black, *n*=8) sheep. Data are mean ± SEM. Roman numerals denote the significant difference between experimental groups (RM ANOVA: *i*, effect of time, p<0.05; *ii*, time*experimental group interaction p<0.05). Symbols denote significant differences amongst experimental groups upon post hoc testing: ‡FGRS vs. FGRI *p*<0.05.

	CON	FGRS	FGRL
Postnatal illness	4°	4°, 1•	4°
Paddock death			1+

Supplementary Table 1. **Animal losses during experimental procedures.** Sheep excluded, euthanised, or found dead postnatally due to: °infection-related deaths (coccidiosis, n=2; eosinophilic gastroenteritis and hepatocellular atrophy, n=1; listeriosis, n=2; lymphocytic meningoencephalitis, n=1; pneumonia, n=4; rhododendron poisoning, n=1; umbilical infection, n=1), •non-specific pulmonary edema, n=1; + fight wounds, n=1.

	Female			Male			Significance (treatment effect)	
	CON *n*=11	FGRS *n*=13	FGRI *n*=13	CON *n*=21	FGRS *n*=10	FGRI *n*=13	Female	Male
BMC (g)	745±45	703±42	698±45	826±83	760±87	827±86	ns	ns
BMD (g·cm^{-2})	1.38±0.05	1.33±0.04	1.26±0.13	1.40±0.10	1.37±0.12	1.42±0.11	ns	ns
Chest LM (kg)	12.3±0.6	12.0±0.6	12.1±0.6	15.8±0.6	14.6±0.7	15.1±0.7	ns	ns
Chest FM (kg)	2.5±0.6	2.1±0.6	2.2±0.6	1.2±0.2	0.7±0.2	0.7±0.2	ns	ns
Abdominal LM (kg)	20.3±1.2	19.5±1.2	19.2±1.2	24.8±0.5	22.8±0.8	23.0±0.8	ns	ns
Abdominal FM (kg)	4.1±1.0	3.4±0.9	3.5±1.0	1.9±0.4	1.1±0.4	1.1±0.4	ns	ns
Rump LM (kg)	8.9±0.4	8.6±0.4	8.9±0.4	10.7±0.4	9.8±0.5	10.2±0.4	ns	ns
Rump FM (kg)	1.8±0.4	1.5±0.4	1.6±0.4	0.8±0.1	0.5±0.2	0.5±0.2	ns	ns
Total LM (%)	41.4±2.2	40.2±2.1	40.2±2.2	51.4±1.4	47.3±1.8	48.4±1.6	ns	ns
Total FM (%)	8.4±2.0	7.0±1.9	7.2±2.0	3.9±0.7	2.3±0.8	2.2±0.8	ns	ns
LM:WT (%)	70.0±1.5	70.5±1.3	73.1±1.7	75.8±1.0	76.7±1.3	76.2±1.2	ns	ns
FM:WT (%)	14.1±2.7	12.4±2.6	12.1±2.6	5.7±1.1	3.4±1.2	3.5±1.2	ns	ns
Chest FM:WT (%)	4.2±0.8	3.7±0.8	3.6±0.8	1.7±0.3	1.0±0.4	1.1±0.4	ns	ns
Abdominal FM:WT (%)	6.8±1.3	6.0±1.2	5.8±1.3	2.7±0.6	1.6±0.6	1.7±0.6	ns	ns
Rump FM:WT (%)	3.0±0.6	2.7±0.5	2.6±0.6	1.2±0.2	0.7±0.2	0.7±0.2	ns	ns
Abdominal FM:Total FM (%)	48.8±0.6	48.5±0.5	47.3±0.7	48.2±0.6	48.3±0.6	48.2±0.6	ns	ns

Supplementary Table 2. **Body composition at 12 months of age.** Bone mineral content (BMC), bone mineral density (BMD) compartmental and total lean mass (LM) and fat mass (FM), absolute and, where appropriate, relative to weight (WT). Data are mean ± SEM.